

## Examination of Thick Blood Films for Malaria Parasites

### Introduction

The definitive diagnosis of malaria infection is still based on finding malaria parasites in blood films. In thin films the red blood cells are fixed so the morphology of the parasitised cells can be seen. Species identification can be made, based upon the size and shape of the various stages of the parasite and the presence of stippling (ie bright red dots) and fimbriation (ie. ragged ends).

**However, malaria parasites may be missed on a thin blood film when there is a low parasitaemia.** Therefore, examination of a thick blood film is recommended. With a thick blood film, the red cells are approximately 6 - 20 layers thick which results in a larger volume of blood being examined.

### Field's stain method for THICK blood films

The method recommended for staining thick blood films by the Hospital for Tropical Diseases is Field's stain which is made from 2 components. Field's A is a buffered solution of azure dye and Field's B is a buffered solution of eosin. Both Field's A and B are supplied ready for use by the manufacturer.

### Method

1. Place a drop of blood on a microscope slide and spread to make an area of approximately 1 cm<sup>2</sup> . It should just be possible to read small print through a thick film.
2. The film is air dried and **NOT** fixed in methanol.
3. The slide is dipped into Field's stain A for 3 seconds.
4. The slide is then dipped into tap water for 3 seconds and gently agitated.
5. The slide is dipped into Field's stain B for 3 seconds and washed gently in tap water for a few seconds until the excess stain is removed.
6. The slide is drained vertically and left to dry.

### Microscopic examination of the Field's stained thick blood film

- The end of the film at the top of the slide when it was draining should be looked at. The edges of the film will also be better than the centre, where the film may be too thick or cracked.
- In a well stained film the malaria parasites show deep red chromatin and pale blue cytoplasm.

- White cells, platelets and malaria pigment can also be seen on a thick film. The leucocyte nuclei stain purple and the background is pale blue. The red cells are lysed and only background stroma remains. The occasional red cell may fail to lyse.
- Schizonts and gametocytes, if present, are also easily recognisable.
- A thick film should be examined for at least 10 minutes, which corresponds to approximately 200 oil immersion fields, before declaring the slide negative.

### Difficulties in the examination of thick blood films

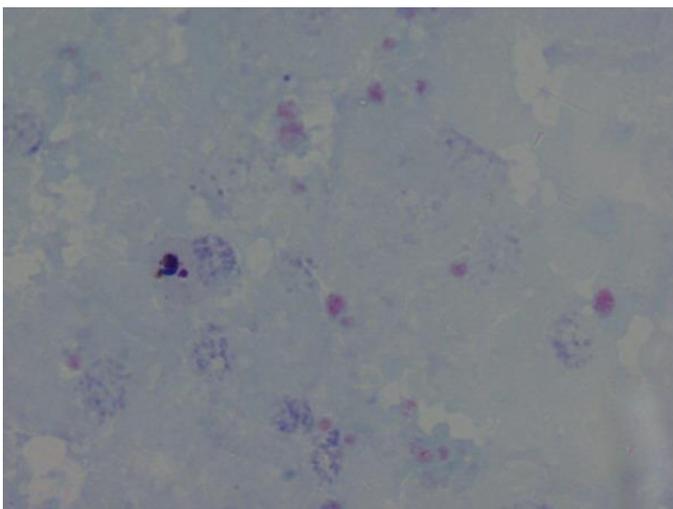
- As a result of haemolysis of the red blood cells due to staining of an unfixed film, the only elements seen are leukocytes and parasites, the appearance of the latter being altered.

Consequently:

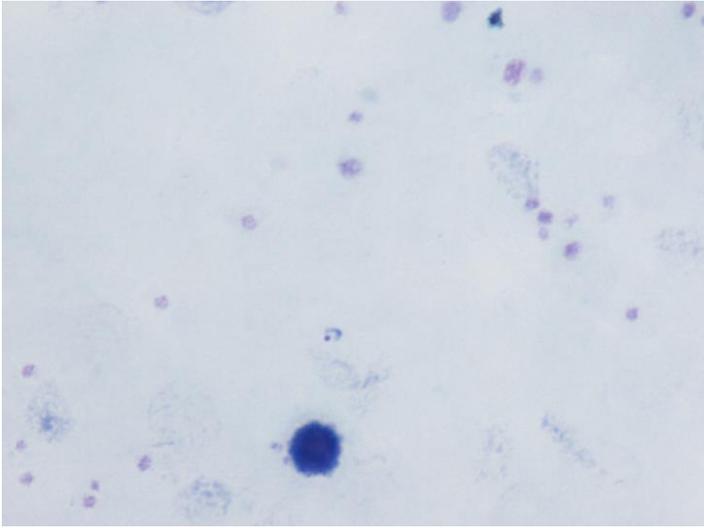
1. The young trophozoites appear as incomplete rings or spots of blue cytoplasm with detached chromatin dots.
  2. The stippling of *P. vivax* and *P. ovale* may be less obvious although occasionally ghost stippling may be seen.
  3. The cytoplasm of late trophozoites of *P. vivax* and *P. ovale* may be fragmented.
- Caution should be exercised when examining thick blood films as artefacts and blood platelets may be confused with malaria parasites.

### Conclusion

A thick blood film is recommended for routine diagnosis of malaria in addition to the thin film and is particularly valuable in instances of low parasitaemia. However, in order to correctly speciate the parasite, examination of a thin film is required. If the thick film is negative, it is unlikely that parasites will be found in the thin film.



*Plasmodium vivax* trophozoite in a thick blood film



*Plasmodium falciparum* trophozoite in a thick blood film